Published as: *Angew Chem Int Ed Engl*. 2013 November 4; 52(45): 11796–11799.

Tailored Glycopolymers as Anticoagulant Heparin Mimetics

Young In Oh,
Division of Chemistry and Chemical Engineering and Howard Hughes Medical Institute, California Institute of Technology, 1200 E. California Blvd, Pasadena, CA 91125 (USA)

Gloria J. Sheng,
Division of Chemistry and Chemical Engineering and Howard Hughes Medical Institute, California Institute of Technology, 1200 E. California Blvd, Pasadena, CA 91125 (USA)

Shuh-Kuen Chang, and
Department of Chemistry and Biochemistry, The Ohio State University, 281 W. Lane Ave, Columbus, OH 43210 (USA)

Linda C. Hsieh-Wilson*
Division of Chemistry and Chemical Engineering and Howard Hughes Medical Institute, California Institute of Technology, 1200 E. California Blvd, Pasadena, CA 91125 (USA)

Abstract

Heparin and its low molecular weight derivatives are clinical therapeutics used to treat and prevent blood clots, but are prone to side effects and contamination. Here we describe the design and expedient synthesis of heparin-based glycopolymers that are potent and potentially safer mimetics of heparin. The mimetics exhibited strong activity against proteases in the coagulation cascade and prolonged blood clot times in human plasma with efficacies similar to those of clinical anticoagulants.

Keywords

glycopolymers; heparin; anticoagulant; antithrombin III; glycosaminoglycans

Heparin, a heterogeneously sulfated glycosaminoglycan, has been used as an anticoagulant drug for over 80 years. Although it is a highly effective and inexpensive agent, heparin in its unfractionated form has several limitations as a therapeutic. First, it is extracted from porcine or bovine mucosa and tissue and carries the potential risk of contamination. The global distribution of contaminated heparin in 2007, for example, resulted in over 100 deaths and hundreds of additional cases reporting adverse clinical effects.[1] Second, heparin displays a variable dose-response relationship among patients, in part due to its structural heterogeneity, and thus often requires active monitoring to fine-tune the dosages.[2] Lastly, approximately 3% of patients undergoing prolonged heparin therapy experience heparin-induced thrombocytopenia (HIT),[3] a severe autoimmune response triggered by formation of a complex between heparin and platelet factor 4 (PF4). Altogether, these factors underscore the critical need to develop safer alternatives to animal-derived heparin that have more predictable bioactivity and reduced side effects.

*Fax: (+1) 626-564-9297, lhw@caltech.edu.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

In recent decades, two forms of heparin-based anticoagulants have emerged as alternatives to unfractionated heparin. Low molecular weight heparin (LMWH) is produced by chemical or enzymatic degradation of heparin, yielding fragments with an average molecular weight of 6000 Da.[4] Shorter ultralow molecular weight heparin (ULMWH; ~1500 Da) is prepared by synthesis[5] of a specific heparin pentasaccharide that represents the minimal epitope required for antithrombin III (ATIII) activation.[6] The commercial drug Arixtra (GlaxoSmithKline; Scheme 1) is a methylated derivative of this pentasaccharide. Addition of another 3-*O*-sulfate group has been shown to confer even greater specificity for ATIII activation (Org31550; Scheme 1).[7] For the prophylaxis and treatment of thromboembolic diseases, LMW and ULMW heparins are preferred over unfractionated heparin due to their more predictable pharmacokinetics, longer half-lives, and reduced risk of side effects.[3] However, animal-derived LMWH still has considerable structural heterogeneity and is susceptible to external contamination.[1b] ULMWH is notoriously difficult and expensive to synthesize (~ 50 chemical steps, ~ 0.1% overall yield in the case of Arixtra)[5a,b] and exhibits limited inhibitory activity toward thrombin (also known as Factor IIa (FIIa)) due to its shorter length.[5a] Thus, despite the notable advantages of LMW and ULMW heparins, new methods are greatly needed for the efficient preparation of defined heparin-like molecules with broad anticoagulation profiles and improved safety.

Synthetic glycopolymers[8] bearing short, well-defined heparin epitopes may offer an attractive alternative to natural heparins and Arixtra. Here, we utilize ring-opening metathesis polymerization (ROMP)[9] chemistry to generate a series of heparin-based glycopolymers and demonstrate that their structures can be tailored to recapitulate the potent activity of anticoagulant drugs. Previously, we showed that glycopolymers containing defined chondroitin sulfate (CS) di- and tetrasaccharide[10] and heparan sulfate (HS) disaccharide[11] epitopes could mimic the activity of natural glycosaminoglycans in neurite outgrowth[10] and immune cell migration[11] assays, respectively. However, the application of ROMP to heparin, the most highly charged and synthetically challenging glycosaminoglycan, has not been investigated. Moreover, synthetic glycopolymers with medicinally significant anticoagulant activity have not been extensively explored as potential heparin mimetics. Our approach overcomes the challenges of heterogeneous sulfation and complex oligosaccharide synthesis and shows promise for the development of a new class of medicinal agents.

The first challenge in designing an anticoagulant glycopolymer was to select a minimal unit that encapsulated the key determinants of the ATIII-binding pentasaccharide. Although previous studies had suggested that a pentasaccharide was the minimum active motif,[5,6] we reasoned that a disaccharide epitope might be sufficient when presented on a multivalent, polymeric scaffold that might enable enhancement of its binding affinity through avidity.[12] Thus, we chose two monosaccharide units (G and H, Scheme 1) from the reducing end of the pentasaccharide, given their well-characterized interactions with the A-helix (R46, R47) and P-helix (K114, D113) of ATIII.[13] The inclusion of unit G allowed us to exploit the conformational flexibility of L-iduronic acid, which substantially improves the affinity of heparin for ATIII.[14] We chose unit H of Org31550 because it contains a rare 3-*O*-sulfate modification that significantly enhances the interaction of heparin with ATIII.[7]

Glycopolymer **1**, comprised of units G and H, can be readily derived from disaccharide **3** (Scheme 2). We devised a protecting group strategy for **3** that would allow for expedient sulfation at four different sites. A benzoyl (Bz) group was used to protect C3, and acetyl (Ac) groups were selected to protect the C2' and C6 positions. These groups can be removed under the same conditions required for C5' saponification[15] to expose simultaneously the three sites desired for *O*-sulfation. In addition, the C2' Ac group was expected to facilitate formation of the 1,2-*trans* glycosidic linkage through neighboring group participation. Due

to the highly charged nature of the sulfated monomer required for polymerization, we elected to use benzyl (Bn) groups at the non-sulfated positions (C3' and C4') to enhance monomer solubility during ROMP.

Orthogonally protected disaccharide **4** was prepared from norbornene-conjugated acceptor **5**[16] and glycosyl phosphate donor **6** (Scheme 3 and Supporting Information (SI) Scheme S1). The coupling reaction utilized stoichiometric TMSOTf at −30 °C and afforded exclusively the α-linked disaccharide **4** in 85% yield. Late stage manipulations to the resulting disaccharide followed precedence from earlier reports.[15,17] Briefly, **4** was subjected to the sequential addition of LiOOH and NaOH to saponify the C5' methyl ester and concomitantly remove the Ac and Bz protecting groups. Global *O*-sulfation of disaccharide **7** using SO_3•NMe_3 in DMF furnished trisulfated intermediate **8** in 71% yield. Finally, the azide group was reduced under Staudinger conditions, followed by regiospecific *N*-sulfation with SO_3•pyr[18] to afford tetrasulfated **3** in 54% yield over two steps.

With monomer **3** in hand, we evaluated a series of polymerization conditions. While polar solvents were required for complete solubility of **3**, unfortunately, such conditions compromised the overall efficiency of the ROMP catalyst. Attempts at aqueous ROMP using a water-soluble catalyst (H_2IMes-poly(ethylene-glycol))$(Cl)_2$Ru=CH(*o*-*i*PrOC_4H_4)[19] led to incomplete conversion and low molecular weight polymers. Polymerization reactions in MeOH using fast initiating catalyst (H_2IMes)$(Py)_2(Cl)_2$Ru=CHPh) **9**[20] produced a similar undesired outcome. Ultimately, we found that polymerization of **3** was fully compatible with a MeOH/$(CH_2Cl)_2$ cosolvent system at 55 °C,[10] resulting in high conversions and good glycopolymer polydispersities. Using this cosolvent system, we then explored the relationship between catalyst loading and polymer length. By tuning the amount of catalyst and MeOH/$(CH_2Cl)_2$ cosolvent ratio, we synthesized a series of glycopolymers with controlled, predictable chain lengths (**10-n**, where n = degree of polymerization (DP); Table 1). Under the tested conditions, polymer lengths were limited to 45 units due to premature precipitation of polymer **10** during ROMP. All glycopolymers were characterized at this stage by ^{1}H NMR spectroscopy and size exclusion chromatography multi-angle light scattering (SEC-MALS). Hydrogenation of the remaining Bn groups using $Pd(OH)_2$/C in a mixture of MeOH and neutral phosphate buffer[21] afforded the desired glycopolymers (**1-n**) in 63–92% yield.

We next assessed the anticoagulant activities of the glycopolymers in comparison to heparin, LMW heparin, and Arixtra. Binding of heparin to ATIII induces a conformational change that enables ATIII to inhibit the serine proteases Factor Xa (FXa) and FIIa.[13] Protease inhibition was measured using chromogenic substrate assays that monitor the release of *p*-nitroaniline (λ_{max} 405) from specific peptide substrates. Consistent with previous reports,[5,22] heparin, LMWH, and Arixtra attenuated FXa activity in the presence of ATIII, with half maximal inhibitory concentrations (IC_{50}) of 16.5 ± 1.2, 526 ± 71, and 11.0 ± 0.1 nM, respectively (Table 2, Figure S1). To our delight, glycopolymer **1–45** showed greater anti-FXa activity compared to the clinical anticoagulants, exhibiting an IC_{50} value of 5.76 ± 0.04 nM (Figure 1A, Table 2). As the polymer length decreased, we observed a significant reduction in anti-FXa activity (IC_{50} = 684 ± 60 and 1470 ± 578 nM for **1–30** and **1–15**, respectively), until no activity was detected (**1–10, 1–6**, and **1–4**; Table 2, Figure S2). We also assessed the contribution of the glucosaminyl 3-*O*-sulfate modification on unit H by comparing the activity of the glycopolymers to that of 3-*O*-desulfated glycopolymer **2–155**.[11]Remarkably, this single alteration in sulfation pattern abrogated the anti-FXa activity, reaffirming the importance of 3-*O*-sulfation and the specificity of the polymer interaction with ATIII (Figure 1A, Table 2). Thus, controlling the sulfation sequence and chain length of the glycopolymer allows for the fine-tuned modulation of ATIII- mediated inhibition at a critical junction in the blood coagulation cascade.

FIIa is activated downstream of FXa in the coagulation cascade and facilitates blood clotting by converting soluble fibrinogen to insoluble fibrin strands. Unlike FXa, which utilizes a specific pentasaccharide sequence, FIIa necessitates a longer, more highly sulfated heparin template to form an inhibitory ternary complex with ATIII.[5a,13] In agreement with this molecular mechanism and previous reports,[5,22] heparin displayed strong anti-FIIa activity (Figure 1B, Table 2; IC_{50} = 11.0 ± 0.1 nM), whereas LMWH and Arixtra had no appreciable activity (Table 2, Figure S1). Notably, we found that glycopolymer **1–45** was 100-fold more potent than heparin at inhibiting FIIa (IC_{50} = 114 ± 1 pM), which is likely due to a greater number of active tetrasulfated motifs and increased polymer length. These results are exciting because they demonstrate that synthetic glycopolymers can be designed to surpass the biological activity of natural glycosaminoglycans. Glycopolymer **1–30** was also active in this assay (IC_{50} = 577 ± 31 nM), and as expected, the shorter polymers (**1–15, 1–10, 1–6**, and **1–4**) and trisulfated glycopolymer (**2–155**) showed no significant activity (Figures 1B and S2, Table 2).

To assess potential interactions with PF4, we evaluated the ability of PF4 to neutralize the anti-FIIa activity of glycopolymers **1–45** and **1–30**. PF4 was added to the glycopolymers or heparin (0.5 – 500 μg/mL) in the presence of ATIII and excess FIIa, and FIIa activity was measured using the same chromogenic assay. Both heparin and glycopolymer **1–45** interacted strongly with PF4, consistent with reports that longer polysaccharides are more likely to engage PF4.[4,23] However, the anti-FIIa activity of glycopolymer **1–30** was only partially neutralized by PF4 (Figure S3), indicating PF4 reactivity associated with HIT can be minimized to some extent by modulating the polymer length.

Finally, we examined the *ex vivo* clotting times of the glycopolymers, with comparison to clinical anticoagulants, using human plasma samples. As the presence of other proteins in complex serum can interfere with anticoagulant activity, this assay represents a more stringent test of anticoagulant efficacy. We measured the activated partial thromboplastin time (APTT) and prothrombin time (PT) of each compound to determine its ability to inhibit blood clotting via the intrinsic and/or extrinsic pathways of the coagulation cascade, respectively.[24] Heparin increased both the APTT and PT for clotting compared to the saline control (Table 2), whereas LMWH and Arixtra at the same concentration increased only the APTT. Notably, the APTT of the glycopolymers could be controlled by varying the polymer length, with a minimum of 10 disaccharide epitopes (**1–10**) required to prolong the APTT. A slight increase to 15 units (**1–15**) endowed the polymer with APTT properties similar to Arixtra, whereas the PT was not appreciably altered in either case. Glycopolymers **1–30** and **1–45** modulated both the APTT and PT, in agreement with their ability to inhibit FXa and FIIa *in vitro*. Remarkably, tuning of the polymer structure produced anticoagulants with unique hybrid properties. While the APTTs of **1–30** and **1–45** were comparable to those of LMWH and Arixtra, their PTs more closely resembled that of heparin. Lastly, as expected, trisulfated glycopolymer **2–155** showed no appreciable change in APTT or PT, reinforcing the importance of the 3-*O* sulfate modification and the potential to tune the selectivity of the glycopolymers toward specific heparin/HS-binding proteins by modulating the sulfation pattern. Overall, the *ex vivo* profile of the glycopolymers not only validates their efficacy in human plasma, but also their ability to replicate the activity profiles of the clinical anticoagulants. Moreover, we find that fine-tuning of the glycopolymer structure can lead to agents with novel properties not found in naturally occurring oligo- and polysaccharides.

Heparin has been used clinically as an anticoagulant since 1935 for treatment and prevention of deep vein thrombosis during surgery, blood transfusion, and renal dialysis. Recent contamination issues associated with animal-sourced heparin have highlighted the importance of developing safer, more homogeneous alternatives to unfractionated heparin.

While structurally-defined oligosaccharides such as Arixtra offer a valuable solution to this problem, they are notoriously challenging and expensive to synthesize. Here we have reported the design and expedient synthesis of homogeneously sulfated glycopolymers based on a tetrasulfated disaccharide motif found in heparin. We demonstrate that these glycopolymers have potent anticoagulant activity, inhibiting the serine proteases FXa and FIIa through potentiation of ATIII and replicating the *ex vivo* plasma clotting activity of the clinical anticoagulants heparin, LMWH, and Arixtra. Most interestingly, we found that systematic alterations in the glycopolymer length and sulfation pattern enabled the fine-tuned modulation of their anticoagulant activity, leading to the creation of new agents with unique hybrid activities distinct from those of both natural and synthetic glycosaminoglycans. These findings demonstrate the potential of this neoglycopolymer approach to control medicinally significant glycosaminoglycan-mediated events and raise the prospect of developing a novel class of anticoagulants through systematic explorations into the glycopolymer structure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Professor Robert H. Grubbs and Dr. Jean Li for their generous donation of the ruthenium catalyst and for helpful discussions. This research was supported by National Institutes of Health grant R01 GM093627-04 (L.H.W.).

References

1. a) Blossom DB, et al. N. Engl. J. Med. 2008; 359:2674–2684. [PubMed: 19052120] b) Guerrini M, et al. Nat. Biotechnol. 2008; 26:669–675. [PubMed: 18437154]
2. Hirsh J, Warkentin TE, Raschke R, Granger C, Ohman EM, Dalen JE. Chest. 1998; 114:489S–510S. [PubMed: 9822059]
3. Warkentin TE, Levine MN, Hirsh J, Horsewood P, Roberts RS, Gent M, Kelton JG. N. Engl. J. Med. 1995; 332:1330–1335. [PubMed: 7715641]
4. Hirsh J, Levine MN. Blood. 1992; 79:1–17. [PubMed: 1309422]
5. a) Petitou M, van Boeckel CA. Angew. Chem. 2004; 116:3180–3196. *Angew. Chem. Int. Ed*. **2004**, *43*, 3118–3133. b) Petitou, M.; Jacquinet, JC.; Choay, J.; Lormeau, JC.; Nassr, M. U.S. Patent. 4,818,816. 1989. c) Xu Y, Masuko S, Takieddin M, Xu H, Liu R, Jing J, Mousa SA, Linhardt RJ, Liu J. Science. 2011; 334:498–501. [PubMed: 22034431] d) Petitou M, et al. Nature. 1999; 398:417–422. [PubMed: 10201371]
6. Choay J, Petitou M, Lormeau JC, Sinay P, Casu B, Gatti G. Biochem. Biophys. Res. Comm. 1983; 116:492–499. [PubMed: 6651824]
7. Van Boeckel CAA, Beetz T, Vanaelst SF. Tet. Lett. 1988; 29:803–806.
8. a) Kiessling LL, Grimm JC. Chem. Soc. Rev. 2013; 42:4476–4491. [PubMed: 23595539] b) Vazquez-Dorbatt V, Lee J, Lin E-W, Maynard HD. ChemBioChem. 2012; 13:2478–2487. [PubMed: 23132748] c) Richards S, Jones MW, Hunaban M, Haddleton DM, Gibson MI. Angew. Chem. 2012; 124:7932–7936. *Angew. Chem. Int. Ed*. **2012**, *51*, 7812–7816. d) Godula K, Rabuka D, Nam KT, Bertozzi CR. Angew. Chem. 2009; 121:5073–5076. *Angew. Chem. Int. Ed*. **2009**, *48*, 4973–4976. e) Collins BE, Blixt O, Han S, Duong B, Li H, Nathan JK, Bovin N, Paulson JC. J. Immunol. 2006; 177:2994–3003. [PubMed: 16920935] f) Sun X, Grande D, Baskaran S, Hanson SR, Chaikof EL. Biomacromolecules. 2002; 3:1065–1070. [PubMed: 12217054] g) Mammen M, Choi SK, Whitesides GM. Angew. Chem. 1998; 110:2908–2953. *Angew. Chem. Int. Ed*. **1998**, *37*, 2755–2794. h) Bovin NE. Glycoconj. J. 1998; 15:431–446. [PubMed: 9881745] i) Manning DD, Hu X, Beck P, Kiessling LL. J. Am. Chem. Soc. 1997; 119:3161–3162.
9. Grubbs, RH. Handbook of Metathesis. Weinheim: Wiley-VCH; 2003.

10. Rawat M, Gama CI, Matson JB, Hsieh-Wilson LC. J. Am. Chem. Soc. 2008; 130:2959–2961. [PubMed: 18275195]
11. Sheng GJ, Oh Y, Chang S, Hsieh-Wilson LC. J. Am. Chem. Soc. 2013; 135:10898–10901. [PubMed: 23879859]
12. a) Kiessling LL, Gestwicki JE, Strong LE. Curr. Opin. Chem. Biol. 2000; 4:696–703. [PubMed: 11102876] b) Pieters RJ. Org. Biomol. Chem. 2009; 7:2013–2035. [PubMed: 19421435]
13. Li W, Johnson DJD, Esmon CT, Huntington JA. Nat. Struct. Mol. Biol. 2004; 11:857–862. [PubMed: 15311269]
14. Das SK, Mallet JM, Esnault J, Driguez PA, Duchaussoy P, Sizun P, Herault JP, Herbert JM, Petitou M, Sinay P. Chem. Eur. J. 2001; 7:4821–4834. [PubMed: 11763451]
15. Orgueira HA, Bartolozzi A, Schell P, Litjens REJN, Palmacci ER, Seeberger PH. Chem. Eur. J. 2003; 9:140–169. [PubMed: 12506372]
16. Lee S, Brown JM, Rogers CJ, Matson JB, Krishnamurthy C, Rawat M, Hsieh-Wilson LC. Chem. Sci. 2010; 1:322–325. [PubMed: 21274421]
17. Tabeur C, Mallet JM, Bono F, Herbert JM, Petitou M, Sinay P. Bioorg. Med. Chem. 1999; 7:2003–2012. [PubMed: 10530949]
18. de Paz JL, Noti C, Seeberger PH. J. Am. Chem. Soc. 2006; 128:2766–2767. [PubMed: 16506732]
19. Hong SH, Grubbs RH. J. Am. Chem. Soc. 2006; 128:3508–3509. [PubMed: 16536510]
20. Scholl M, Ding S, Lee CW, Grubbs RH. Org. Lett. 1999; 1:953–95. [PubMed: 10823227]
21. Hu Y, Lin S, Huang C, Zulueta MM, Liu J, Chang W, Hung S. Nat. Chem. 2011; 3:557–563. [PubMed: 21697878]
22. a) Chen J, Jones CL, Liu J. Chem. Biol. 2007; 14:986–993. [PubMed: 17884631] b) Sebastian H, Parisius J, Picard-Willems B. Thromb. Res. 2008; 123:396–403. [PubMed: 18571698]
23. Lane DA, Denton J, Flynn AM, Thunberg L, Lindahl U. Biochem J. 1984; 218:725–732. [PubMed: 6721831]
24. Bentorp E, Salvagno GL. Semin. Thromb. Hemost. 2008; 34:670–682. [PubMed: 19085768]

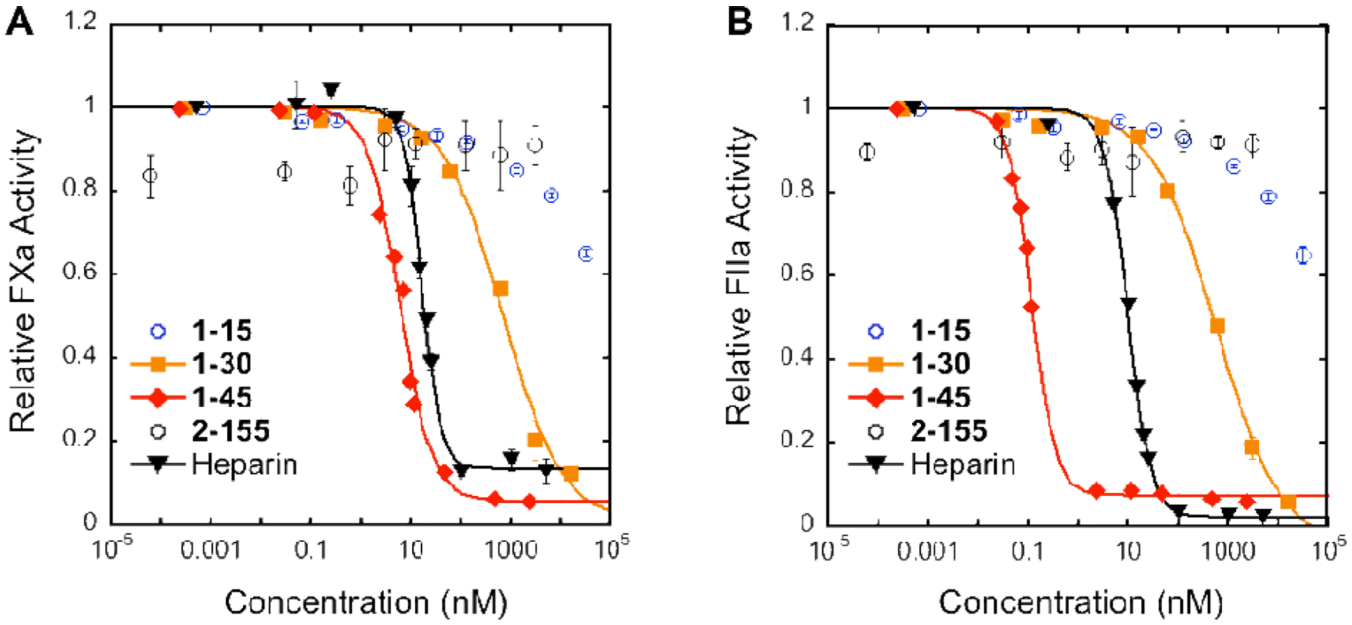
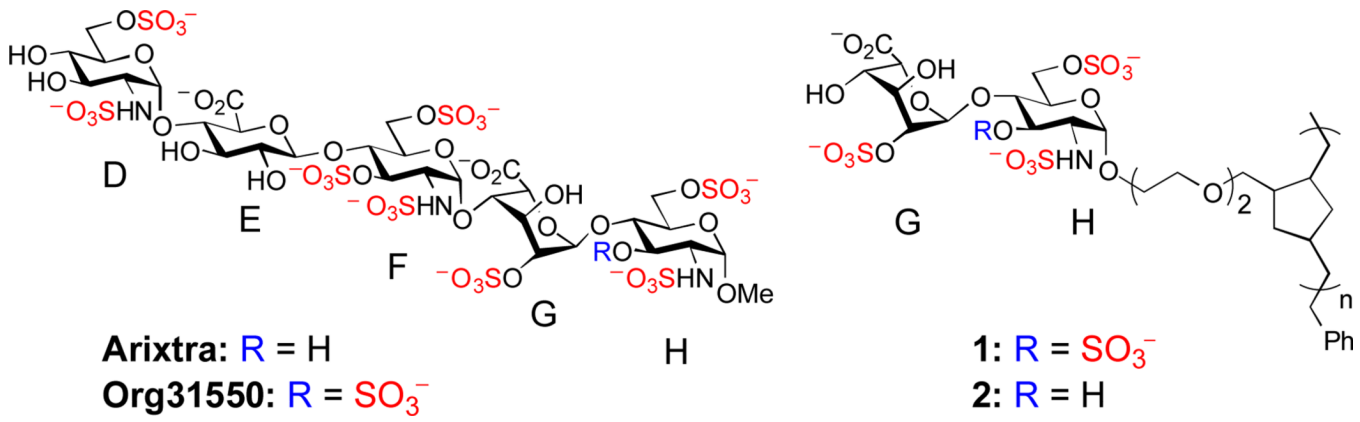
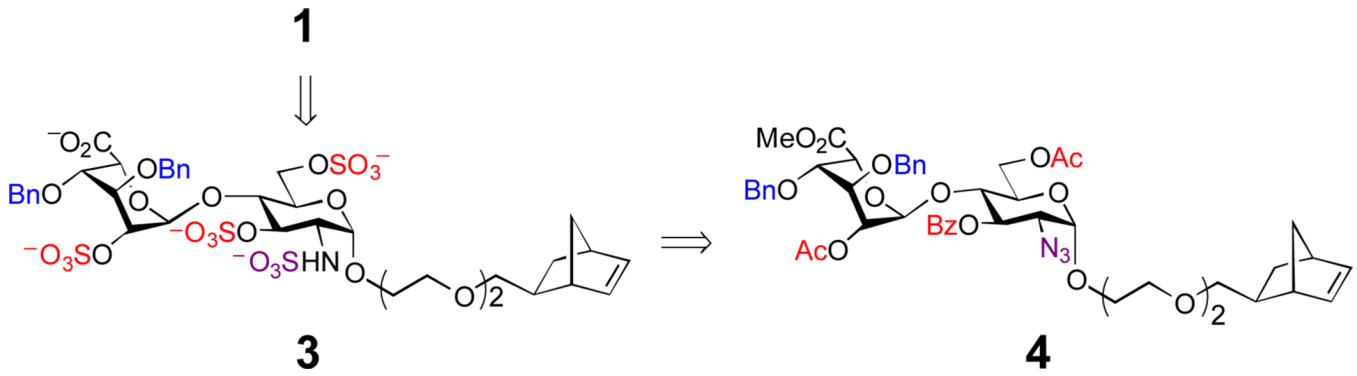


Figure 1.

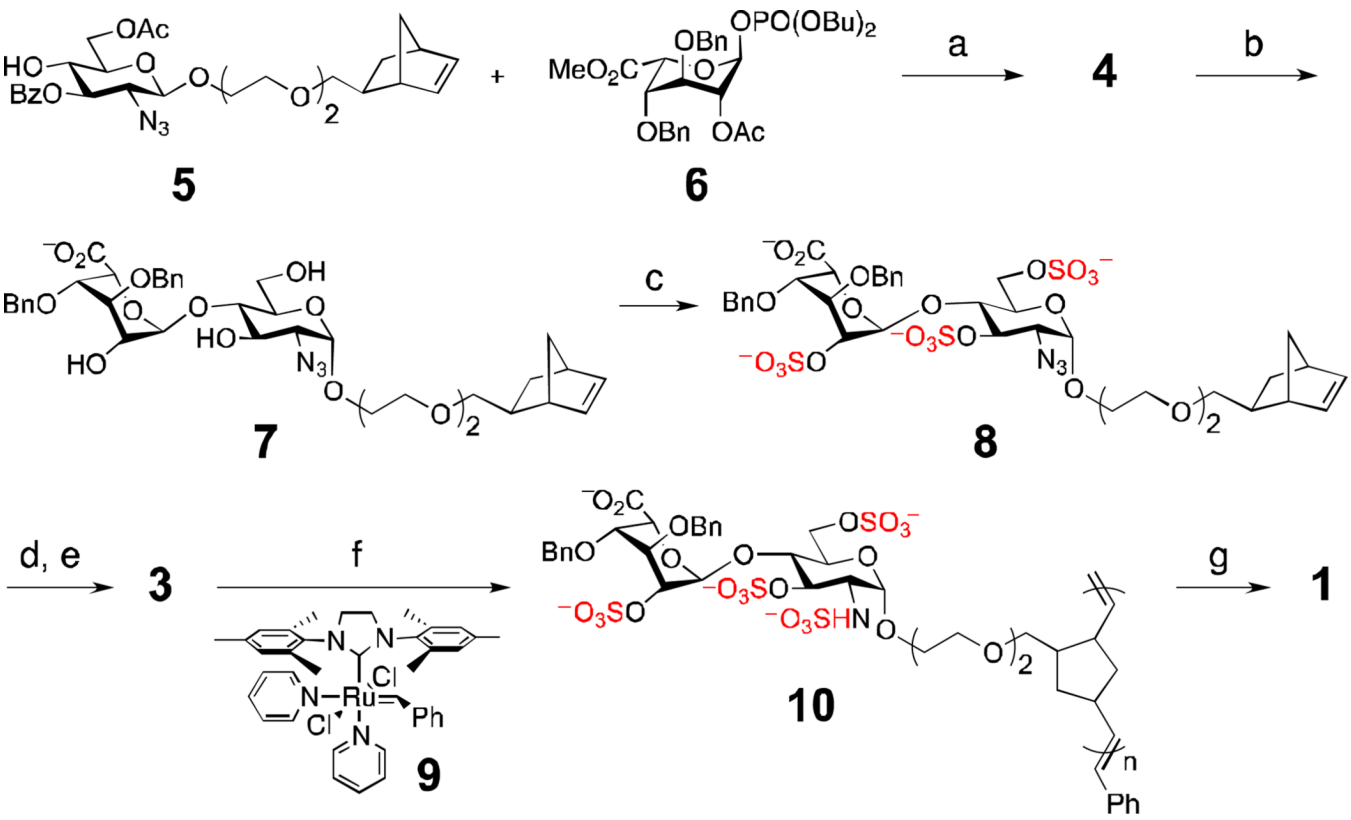
Tetrasulfated glycopolymers (**1–15, 1–30, 1–45**) inhibit the ATIII-mediated (A) FXa and (B) FIIa activity in a length and sulfation-dependent manner. Chromogenic substrate assays were used to measure coagulation factor activity in the presence of ATIII and glycopolymer. Data represent the mean ± standard deviation for n=3 experiments. See Table 2 for IC_{50} values.



Scheme 1.
Chemical structures of Arixtra, Org31550, and synthetic glycopolymers **1** and **2**.



Scheme 2.
Retrosynthesis of glycopolymer **1** from orthogonally-protected disaccharide **3**.



Scheme 3.

Synthesis of tetrasulfated heparin glycopolymer **1**a

[a]Conditions: (a) TMSOTf, CH_2Cl_2, −30 °C, 85%. (b) 0.7 M LiOH, H_2O_2, 25 °C; MeOH, 4 M NaOH, 25 °C, 82% over two steps. (c) SO_3•TMA, DMF, 60 °C, 71%. (d) PMe_3, THF, 25 °C, quant. (e) SO_3•pyr, pyr/Et_3N, 60 °C, 54% over two steps. (f) MeOH:$(CH_2Cl)_2$, 55 °C. (g) $Pd(OH)_2$/C, 1 atm H_2, MeOH, phosphate buffer (pH 7.4), 63–92%.

Table 1

Heparin Glycopolymer Properties

Entry	Polymer	Mol % 9	MeOH: $(CH_2Cl)_2$	n (DP)	M_n (g/mol)	PDI
1	**10-4**[a]	30	1:4	4	4,373	2.03
2	**10-6**[a]	17	1:4	6	6,167	1.25
3	**10-10**[a]	10.7	1:3	10	11,207	1.29
4	**10–15**[a]	6.5	1:3	15	15,452	1.32
5	**10–30**[a]	5.2	1:2.5	30	32,721	1.41
6	**10–45**[a]	2.0	1:2.5	45	42,970	1.25
7	**2–155**[b,c]	4.0	1:10	155	164,345	1.62

Degree of polymerization (DP), number average molecular weight (M_n), and polydispersity index (PDI) were determined by SEC-MALS in

[a] 0.2 M LiBr in DMF or

[b] 100 mM $NaNO_3$, 200 ppm NaN_3 in H_2O.

[c] See Ref 11 for the synthesis of **2–155**.

Table 2

Biological Activity of Heparin Glycopolymers

Polymer	Anti-FXa IC_{50} (nM)	Anti-FIIa IC_{50} (nM)	APTT[a] (s)	PT[a] (s)
1–4	>2000	>2000	032.5 ± 0.3	13.2 ± 0.1
1–6	>2000	>2000	032.2 ± 0.2	13.2 ± 0.3
1–10	>2000	>2000	059.6 ± 0.3	15.8 ± 0.4
1–15	1470 ± 578	>2000	082.9 ± 0.6	23.4 ± 1.9
1–30	684 ± 60	577 ± 31	100.8 ± 0.6	50.8 ± 6.3
1–45	5.76 ± 10^{-2}	0.114 ± 10^{-4}	119.4 ± 0.5	52.2 ± 7.8
2–155	>2000	>2000	046.1 ± 0.4	12.7 ± 0.1
None	>2000	>2000	031.2 ± 0.3	13.3 ± 0.1
Heparin	16.5 ± 1.2	11.0 ± 0.1	>180	84.2 ± 18.
LMWH	526 ± 71	>2000	0.117 ± 3.0	14.8 ± 0.2
Arixtra	11.0 ± 0.1	>2000	078.3 ± 0.4	15.1 ± 0.3

[a] 150 μg/mL compound in citrated human plasma, *n=3*